Progress Report

# PHYSICS OF CELLULAR SYNTHESIS, GROWTH AND DIVISION

NsG-324

(Period Covered: July 1, 1966 - December 31, 1966)



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## I. INTRODUCTION

The following status report covers the first six months of the 1966-67 budget period of NASA contract NsG-324. Because of the change in the starting date this year from October 1 to July 1, the last few months of the preceding budget period overlapped the first months of this period. Consequently, much of the work carried out during the past six months was covered in the final status report for 1965-66 (dated November 7, 1966). This report includes a brief summary of the studies already described in detail in the November report and four reports on investigations which were not covered in November.

As indicated in the previous report, hyperchromicity and density gradient studies have given good evidence that ionizing radiation causes double-strand breakdown of E. coli DNA. The first of two papers on these studies was referenced in that report and the second has now been published (1).

Density gradient centrifugation studies have also been applied to living cells and preliminary results have shown that intact bacterial cells can be banded in a CsCl density gradient with a portion of the population surviving. A paper reporting these studies is in press with the Biophysical Journal (3). Centrifugation studies have shown also that irradiated E. coli cells can be differentiated from unirradiated control cells in the CsCl density gradient system. The centrifugal studies to be published in Biophysical Journal and other investigations of the effect of high centrifugal forces on macromolecular synthesis were detailed in the last report. Additional studies

are now in progress examining the alteration of banding position by gamma-irradiation and comparing these studies with "leakiness" studies also in progress in the laboratory (Section IIB).

In other centrifugation studies the effect of centrifugal forces on the synthetic processes in bacteria are being studied. Ficoll, a high molecular weight polysaccharide, is added to the medium to keep the cells from forming a pellet during centrifugation. Studies of the uptake of labeled components indicate that a force of 50,000g has little effect on DNA, RNA and protein synthesis (Section IIA).

Experiments are also in progress investigating the mechanism of T4 phage replication in ultraviolet light-induced filamentous E. coli cells (Dr. Ginoza). These studies indicate that the parental DNA occupies only a small region in the bacterial cell during the latent period and that the progeny DNA do not migrate far inside the cell. This data is presented more fully in Section IIC.

Current electron spin resonance studies of gamma-ray-induced free radicals in organic solids were detailed in the November status report. These included investigations of radicals produced by gamma-irradiation and hydrogen atom bombardment of amino acids. Gamma-irradiated thymine, dihydrothymine, and thymine dimer were also studied. In addition, the E.S.R. studies of free radicals produced by tritium decay and certain dyes are being continued. Results which indicate that the saturation level reached in free radical concentration is due to free radical turnover have led to attempts, now in progress, to relate the rate constants of different compounds to their crystalline structure. Two papers are in press with Radiation Research and the Journal of Chemical Physics (4, 5).

In Dr. Strother's laboratory microspectrophotometric studies of cellular components are progressing. Instrumentation improvement continues and a complete description of changes made during the past year was given in the

November status report.

The mutation model described in the last status report by Dr. Person's group is being used to examine the base changes resulting from several different mutagens; and the effects of high sugar concentrations on bacterial growth which were discussed in that report (Dr. Scheie) are being investigated further. Studies of the denaturation kinetics of T4 DNA and of methods of observation of whole bacterial chromosomes are continuing also (Dr. Taylor). These lines of research were all covered in some detail in the last status report.

The mammalian cell culture laboratory has been completed. A report on the research now in progress is given in section IID.

The combined results of the work during the past six-month period have suggested a few more clues toward understanding the complex functioning of the cell, the ultimate goal of this program. The range of studies which is possible in a diversified program of this type allows several approaches to the same problems. For example, the genetic effects of a mutagenic agent can be studied in the genetics laboratory at the mutational level while the actual molecular effects can be examined by electron spin resonance. Having one major area of support and, essentially, one basic aim, understanding cellular processes, keeps this program integrated. This NASA contract continues to serve as the unifying core of the program.

## II. INDIVIDUAL REPORTS

### A. Centrifugation of E. coli Cells.

E. C. Pollard.

Although a considerable amount of work has been devoted to the observation of centrifuged cells and their recovery after centrifugation, notably by Zalokar, the very important question as to whether the cells function while they are being centrifuged has not been answered. It was thought worthwhile to devise an experiment which would enable the study of the synthetic processes of cells while in the distorted condition due to centrifugation.

In order to do this a polysaccharide material of high molecular weight, ficoll (Pharmacia), was used. This is soluble in almost any amount of medium, certainly up to 50%. It is inert with regard to trichloracetic acid and it readily passes through filters which will hold back bacteria. In view of this it seemed a very suitable material for producing a density equivalent to that of cells thus permitting centrifugation to be applied without actually forcing the cells to form a pellet.

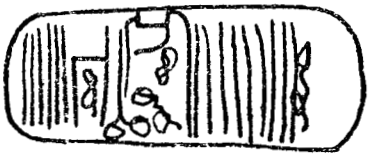
The concept of the experiment is indicated in the first figure. Cells are supposedly centrifuged into a distorted condition under 50,000g. The hope would be that under these conditions the DNA and polysomes should sediment to one end, resulting in a reduction in RNA and DNA synthesis, and possibly even in protein synthesis.

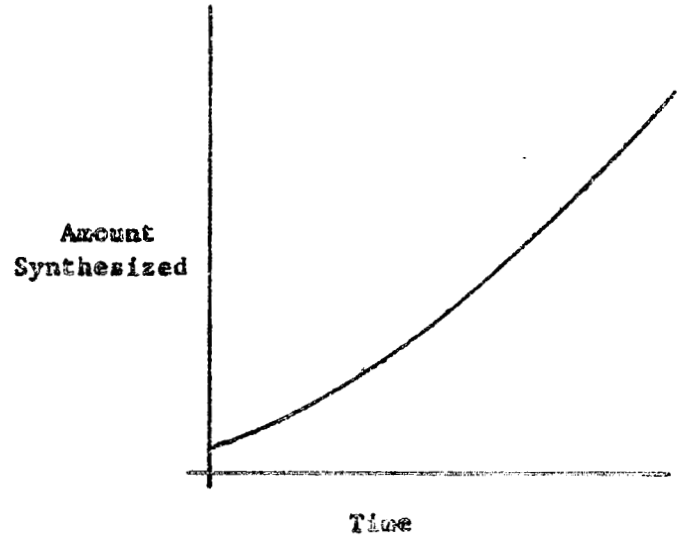
The internal sedimentation velocities of cellular components can be calculated using the following equations:

$$\text{Force} = \frac{4}{3}\pi a^3(\rho - \rho_o) \times \text{"g"}$$

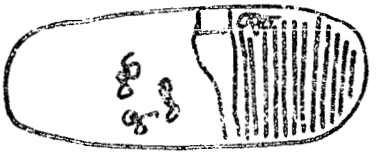
$$\text{Resistance} = 6\pi\eta av$$

A. Normal cell






B. Cell at 50,000g



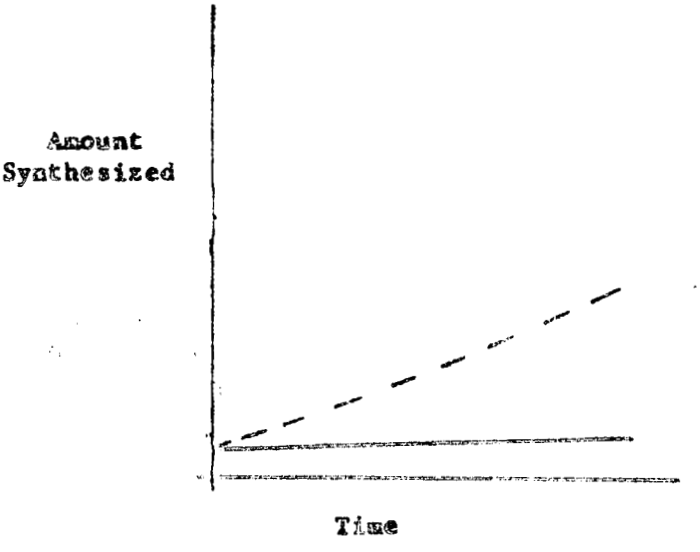



Figure 1. Concept of experiment. Under centrifugation the DNA and polysomes should sediment to one end, reducing RNA and DNA, and possibly protein, synthesis.

For the DNA "nucleus" we assume:

$$a = 2200 \text{Å}$$

$$\frac{\rho}{\rho_0} = 0.1$$

$$50,000 \frac{\text{g}}{\text{cm}^3}$$

$$\eta = 10 \text{ poise}$$

the velocity is calculated to be

$$v = 10^5 a^2 \text{ cm/sec}$$

$$v = 4 \times 10^{-5} \text{ cm/sec}$$

Thus, the nucleus should reach the end of the cell in a few seconds. Using the same equations polysome sedimentation can be calculated also.

Assume $\frac{\rho}{\rho_0} = 0.2$ thus

$a = 4 \times 10^{-6}$ cm $\quad v = 3.9 \times 10^{-5}$ cm/sec

$\eta = 1$ poise*

The internal sedimentation of the DNA "nucleus" and the polysomes are almost identical. However, the question remains as to whether the cell contents undergo Brownian movement during centrifugation. The following calculations can be made to determine the probability of this happening:

$$\delta^2 = 2Dt = \frac{2kT}{\xi} t = \frac{2kT}{6\pi\eta a} t$$

For the "nucleus" - $\delta^2 = \frac{2 \times 4.2 \times 10^{-14}}{6\pi \times 10 \times 2 \times 10^{-5}}$

$\delta = 5 \times 10^{-5}$ per second

For the polysomes $\eta = 1$, $a = 4 \times 10^{-6}$

$\delta = 5 \times 10^{-5}$ cm/sec

This indicates that the "nucleus" should not have different but that the polysomes might form an internal gradient by diffusion.

The data obtained with these experiments are presented in Table I, A and B.

* suggested by earlier data obtained for tubulin and ribosomes.

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It can be seen that with the sole exception of the case of leucine uptake there is very little observed effect on synthetic processes. DNA and RNA synthesis appear to be unaffected. Temperature variation could account for the increased uptake in the centrifuged cells. The lower value for uptake of leucine by centrifuged cells was repeated in 4 experiments and is probably significant. It indicates that some aspect of protein synthesis may be slightly influenced by 50,000g, although this effect is slight.

It could be argued that cells tumble during centrifugation. The following calculation indicates that this is unlikely:

Rotational Brownian Movement

$$\theta^2 = \frac{2kTt}{8\pi\eta R^3}$$

$R = 10^{-4}$ cm

$\eta = 10$ poise

$T = 300^{\circ}K$

$k = 1.4 \times 10^{-16}$ erg/°K

For $\theta = 2\pi$

$$t = \frac{16\pi^3 R^3 \eta}{kT} \quad ; \quad t = 1.2 \times 10^5 \text{ sec}$$

Experimental data corroborate this suggestion. Filamentous cells, formed by treating cells with ultraviolet light to give cells up to 50 microns in length, were subjected to continuous sedimentation under similar conditions. No observed change in the uptake of amino acids was observed.

The conclusion from the data at present is that either centrifugation does not force a rearrangement of the internal structure of the bacterial cell, even at forces as high as 50,000g, or the cell has the ability to function in spite of gross distortion.

Future experiments are planned to expand the studies above in two ways. The first is to use larger cells in which there should be more total effect. Second, we plan to study the effects of centrifugal forces higher than 50,000g.

B. CsCl Density Gradient Centrifugation of Bacterial Cells.

E. C. Pollard and C. E. Hildebrand.

A major portion of the work being done in this area is directed toward a study of the effect of radiation ($Co^{60}$-gamma rays) upon banding position of bacterial cells (*E. coli* $B_{s-1}$ and *E. coli* $T^-L^-$) in a cesium chloride density gradient. Preliminary experiments indicate that gamma-irradiation does alter the banding position. The intent of present investigations is to demonstrate a correlation between the measured change in banding position of irradiated cells in relation to (1) unirradiated (control) cells and (2) the expected change, based upon the amount of leakage of various components from the cell (e.g. DNA, RNA's, protein, carbohydrate and lipid) determined by independent methods. Leakage studies are being done by Dr. E. C. Pollard and Patricia K. Weller. Thus far, experimental data indicate that such a correlation can be made if it is assumed that all cellular components are solvated in the cesium chloride density gradient.

Future experiments will include a verification of the data already in hand, a study of the effect of different radiation doses upon change in banding position and a study of the change as a function of time after irradiation.

C. T4 Replication in Filamentous *E. coli*.

W. Ginoza.

Studies are in progress in this laboratory on the mechanism of T4 replication in ultraviolet light-induced filamentous *E. coli* cells, using radioautographic technique and genetic recombinational analysis. T4 DNA is 60 $\mu$ in length. Results thus far show that the injected phage DNA (labeled with tritium) occupies a very small region (3 - 5 $\mu$) in the host cell during the entire latent period, despite the extended space available in the filamentous cells (31 $\mu$, average). Frequencies of recombination between two strains of amber mutants in these non-permissive host cells have been tested.

These are shown to decrease markedly with increasing length of the filaments. Since recombination can occur only by direct physical contact between two interacting genomes, the result confirms the lack of spread of the parental DNA seen by autoradiography. Moreover, it seems to indicate that progeny DNA also do not migrate much. Indeed, some single bursts yield populations of progeny consisting only of the two parental types and no recombinants. The combined results suggest that fragmentation of vegetative DNA is not a necessary prelude to genetic recombination nor to multiplicity reactivation.

D. Effects of Radiations on Cultured Mammalian Cells.

P. Todd, H. Dalen, A. Hellewell.

Since the last report, the cell culture laboratory has been completed and research projects have commenced. We have accumulated evidence confirming that "lipovirus infected human liver cells" (Little and Chang, Science 148; 1746 , 1965). which are characterized by extreme radiation resistance, among other unusual features, are amoebae. A semi-automatic, non-traumatic method for harvesting large numbers of synchronous cells from monolayers is under development. Diploid and tetraploid hamster cell lines isolated from small colonies developed from X-irradiated cultures are under study. Evidence has been found that ultraviolet light may induce the same type of inherited radiation injury as does ionizing radiation. This has been the only effect of ultraviolet we have found so far that is similar to any due to ionizing radiations. Further similarities and differences between the two are being sought with the goal of sorting out the characteristics of the "lesions" inflicted.

## III. PUBLICATIONS

One paper has been published since the last report and four more are currently in press. These are listed below. Several papers either are in preparation or have been submitted for publication. In addition, abstracts will be presented at both the Biophysical Society meetings in February and the Radiation Research Society meetings in May.

1. J. Swez and E. C. Pollard. DNA-Agar Annealing of Residual DNA After Degradation by Ionizing Radiation. Radiation Research 29, 475-482 (1966).
2. E. C. Pollard and T. F. Barone. The Effect of Ionizing Radiation on Genetic Transcription: Aspects of the Mechanism. Radiation Research, Suppl. 6, in press.
3. E. C. Pollard and L. J. Grady. CsCl Density Gradient Studies of Intact Bacterial Cells. Biophys. J., vol. 7, in press.
4. W. Snipes and P. K. Horan. Electron Spin Resonance Studies of Free Radical Turnover in Gamma-Irradiated Single Crystals of Alanine. Radiation Research, in press.
5. W. Bernhard and W. Snipes. ESR of Free Radical Conversion in Gamma-Irradiated Dihydrothymine. J. Chem. Phys., in press.

## IV. PERSONNEL

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|---|---|
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| W. Taylor | |
| P. Todd | |
| H. Dalen | Research Associates |
| S. Stein | |
| T. Nevalainen | Post Doctoral Fellow |
| P. Bernhard | Research Assistants |
| B. Nichols | |
| L. Ritchie | |
| M. Trask | |
| W. Bernhard | Graduate Assistants |
| D. Chapman | |
| T. Coohill | |
| J. Elder | |
| C. Gay | |
| L. Grady | |
| R. Hallett | |
| A. Hanks | |
| L. Harris | |
| A. Hellewell | |
| G. Kantor | |
| D. Kearns | |
| M. Mogus | |
| M. Osborn | |
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| A. Yayanos | |
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| | Technical Assistants (Parttime) |